

Ovipositor morphology and egg laying behaviour in the dragonfly *Lestes macrostigma* (Zygoptera: Lestidae)

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(Received 24 July 2010; final version received 18 November 2010)

Lestes macrostigma is a stenotopic dragonfly species of Western Palaearctic distribution that has high conservation status almost throughout its range. It inhabits mainly brackish water with a typical plant species, sea club-rush *Bolboschoenus maritimus*. Due to the absence of special investigations, the nature of this insect–plant association is not clearly understood, but it was supposed that *L. macrostigma* prefers egg laying in *B. maritimus*. In this paper we describe the ovipositor morphology and the egg laying behaviour of *L. macrostigma* in detail. The cutting ovipositor reveals several morphological peculiarities recorded previously in other lestids. The internal surface of the valves reveals rich microsculpture. Numerous single and clustered sensilla of different shape are found on the valves and styli and are probably involved in oviposition–plant recognition by females and/or in production of an egg clutch. Oviposition is carried out in stems of *B. maritimus* and *Juncus maritimus*. An egg clutch consists of a row of single eggs deposited in line along the long axis of a plant. Results are discussed in the light of possible morphological and behavioural adaptation to oviposition into specific plant substrates.

Keywords: Odonata; dragonfly; Zygoptera; Lestidae; *Lestes macrostigma*; ovipositor morphology; egg laying; egg clutch

Introduction

The act of egg laying is very important for the continuation of nearly all insect species. As insects with aquatic larvae, dragonflies face a special problem of detecting suitable oviposition sites within the aquatic environment. Eggs must be deposited in an area that protects them from possible predators, provides suitable environmental conditions for egg development, and provides newly hatched larvae with ready access to water and nutrition.

In Zygoptera, females lay eggs mainly in plant tissues using their well-developed cutting ovipositor (Rostand, 1935). There is evidence that some dragonfly species show clear preferences in selecting plant species or even parts of plants for oviposition (Bick & Bick, 1970; Grunert, 1995; Martens, 1992, 1993, 1994; Robert, 1958; Wildermuth, 1993). Egg laying in specific substrates requires adaptations such as an ovipositor of certain mechanical properties, which is equipped with receptors for exploring suitable sites (see e.g. Matushkina & Gorb, 2002a,

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2007). However, dependence of ovipositor structure in dragonflies on the egg-laying habit is still insufficiently studied.

This paper aims to describe thoroughly the ovipositor morphology and egg laying in *Lestes macrostigma* (Eversmann, 1836). This is a stenoecic species that occurs mainly around brackish water (Jödicke, 1997; Plattner, 1967; Robert, 1958). Whereas the natural habitat of *L. macrostigma* covers a wide region of the South Palaearctic from the Mediterranean to Siberia and the Central Asia, the regional distribution of this species is patchy and very local in most areas (Boudot et al., 2009; Dijkstra & Lewington, 2006; Jödicke, 1997; Sahlen et al., 2004). An important aspect of the association of *L. macrostigma* with brackish waters is the usual presence of a characteristic coastal plant, sea club-rush *Bolboschoenus maritimus* (L.) Palla. It was supposed that adults of *L. macrostigma* not only prefer this plant for oviposition, but also used them to identify the waters that are suitable for larval development (Jödicke, 1997). However, special investigations of choice of the oviposition plant by *L. macrostigma* as well as of its habitat selection are absent. In spite of its strong conservation status from European to regional scales, *L. macrostigma* is still poorly studied and, with regard to conservation, increasing the knowledge of its biology is of primary consequence (Dupont, 2009; Lambret et al., 2009).

Materials and methods

Morphology

Adult females of *Lestes macrostigma* were collected by Alex Gumovsky from South Ukraine (Kherson Province, Golopristsansky District, surroundings of village Rybal'che, saga, 15–29 June 2005; 46°28' N/32°14' E). Fifteen females were examined. Terminal abdominal segments were dissected in median (13 specimens) and frontal (two specimens) planes. The musculature was examined in water under a stereo microscope by manual dissections and subsequent layer-by-layer reconstructions of the muscles. Then cuticle parts were macerated in 10% KOH, examined in glycerine under a stereo microscope Olympus C41 and photographed with an Olympus C4040 camera, using Olympus DP-Soft Version 3.2. For a SEM study the cuticular parts of three females were washed in distilled water, dehydrated in a graded ethanol series and in acetone, critical point dried (OM CPD 7501), sputtered with gold-palladium (OM-SC7640) and examined with a Zeiss EVO-50 SEM. A general description of the odonate endophytic ovipositor was provided by Matushkina (2008).

Oviposition behaviour and measuring of egg clutch

Oviposition was studied during June and July 2008 and in June 2009 in the Marais du Vigueirat (43°32' N/04°45' E, Camargue, France). This site is a protected area belonging to the Conservatoire du littoral and covers 1200 ha that are mainly composed of marshes. *Lestes macrostigma* breeds in temporary brackish pools, where females oviposit in *Bolboschoenus maritimus* and in *Juncus maritimus* Lam. Egg laying behaviour was studied on sunny and calm days (i.e. clouds covering less than 50% of the sky and wind speed up to 5.5 m/s) from 11:00 am to 03:00 pm by direct observations made with binoculars (Zeiss Victory 10 × 42 T*FL) and reconstruction afterwards from videos and pictures made with a Nikon D90 camera. Twenty-two females making 708 incisions in substrate were observed. One tandem was filmed ovipositing in *J. maritimus* and another lone female was filmed ovipositing in *B. maritimus*; those two films allowed us to study substrate examination behaviour and the position of the female abdomen during substrate penetration. Four stems of *J. maritimus* bearing egg clutches of *L. macrostigma* were studied. Egg clutches were measured with a Zeiss Stemi 2000 stereomicroscope equipped with a micrometric ocular.

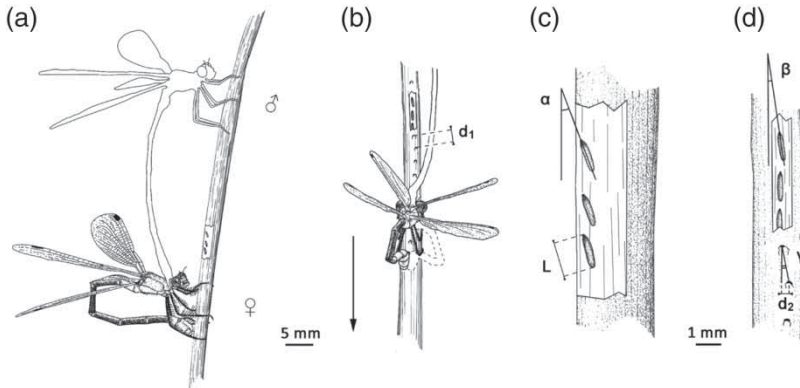


Figure 1. Egg laying of *Lestes macrostigma* in the sea rush, *Juncus maritimus*, and schema of clutch measuring. Drawings from photos: (a) lateral view of tandem with ovipositing female, egg clutch is partly uncovered; (b) dorsal view of ovipositing female that swung and rotated its abdomen from side to side during insertion of the ovipositor, egg clutch is partly uncovered, d_1 is distance between two successive incisions; (c) dissection of an egg clutch in lateral view, α is the angle between the egg axis and the plant surface; (d) partial dissection of an egg clutch in dorsal view, β is the angle between egg axis and the line running parallel to the direction of the plant fibres, γ is the angle between the line connecting two successive insertions and the fibre direction of the plant tissue, d_2 is shortest linear displacement of the egg from the line running through the previous egg parallel to the direction of the plant fibres. Arrow shows backward movement of ovipositing female.

Additionally, plant tissues were partly removed and the position of each egg was photographed in sagittal and frontal planes with the Nikon D90. The location of the egg within plant tissue was measured from pictures. The following parameters were measured (Figure 1): (1) egg length, L ; (2) distance between two successive eggs, d_1 ; (3) shortest linear displacement of the egg from the line running through the previous egg parallel to the direction of the plant fibres, d_2 ; (4) the angle α between egg axis and the plant surface; and (5) the angle β between egg axis and the line running parallel to the direction of the plant fibres. From parameters (2) and (3), the angle γ between the line connecting two successive eggs and the fibre direction of the plant tissue was calculated. All measurements are given by mean \pm standard deviation, if not otherwise stated. Linear measurements are given in mm. Positive or negative value of angular measurements in β and in γ signifies deviation to the right or to the left, respectively. The statistics were analysed using the Past 2.04 software package (Hammer et al., 2001). The Shapiro–Wilk W test was used to test the normality of the data. Non-normally distributed data are characterized by median and range, and a Mann–Whitney U test was used to test differences between two groups.

Abbreviations

a	medial apodeme of basal plate of ovipositor
aAp	anterior apophysis of V3
b	lateral apodeme of basal plate of ovipositor
c	anteromesal apodeme on anterior carina of 9th tergite
d	anterodorsal apodeme on anterior carina of 9th tergite
Ga	gonangulum
Lam	basal plate of ovipositor (lamina valvarum)
M1–M8	muscles of ovipositor
pAp	posterior apophysis of V3
piv	internal sclerite, or posterior intervalvula
PS9	poststernum of 9th segment
St	stylus
V1/V2/V3	first/second/third valves of ovipositor (valvulae 1/2/3)

Results

Morphology of ovipositor

Cutting valves

Cutting valves slightly curved dorsad, with expanded bases (Figures 2–4). Paired V1 fused in most basal region. Dorsal margin of each V1 forming a longitudinal groove (aulax), providing a sliding articulation (olistheter) with V2 (Figure 3a, c). External surface of each V1 with five or six transverse ridges in distal half, slightly enlarged in apical direction. Lateral margins of apices of both V1 slightly serrated (Figure 3a, c). Numerous coeloconic and campaniform sensilla scattered on external surface of V1. Basis of V1 with two clusters of differently directed campaniform

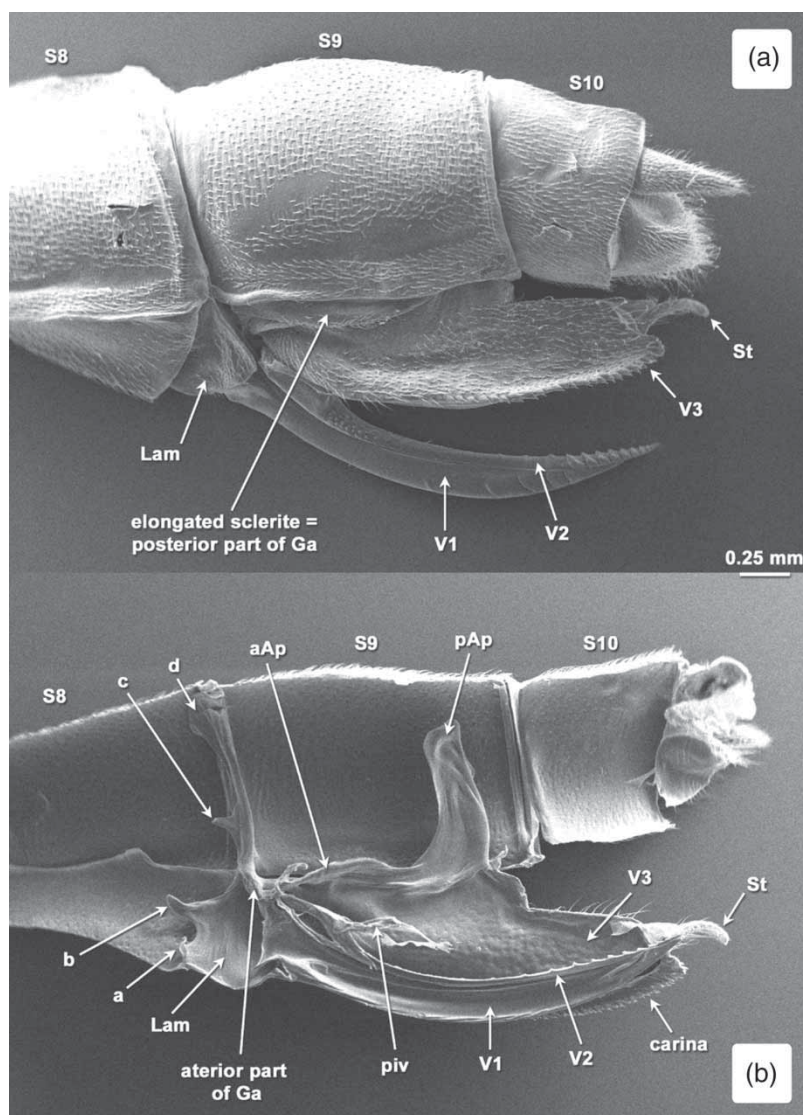


Figure 2. Scanning electron micrographs of the ovipositor of *Lestes macrostigma*: (a) lateral (external) view; (b) medial (internal) view.

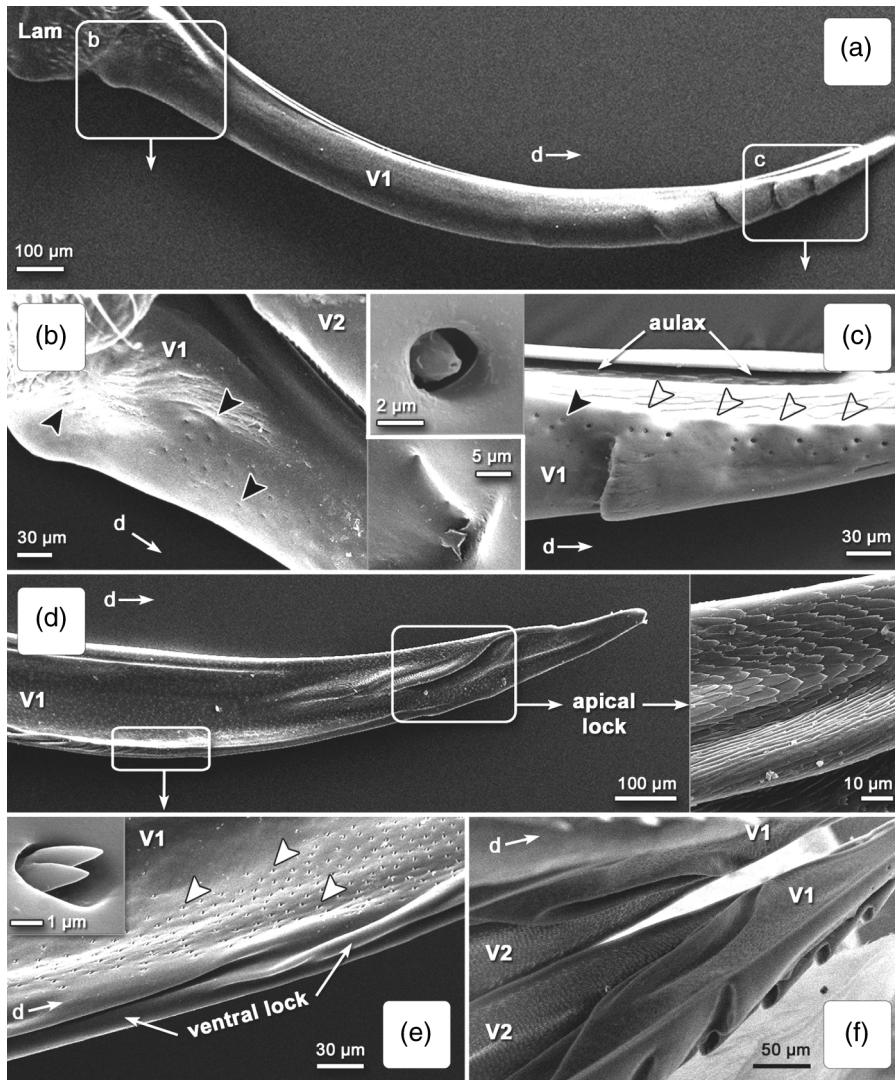


Figure 3. Scanning electron micrographs of the first cutting valve of *Lestes macrostigma*: (a) general view, insets indicate positions of (b) and (c); (b) lateral view of the basal part, inset shows campaniform sensillum; (c) lateral view in area of apical ridges and serrations, inset shows single sensillum; (d) internal view of the apical half, insets show microsculpture of "apical lock" and position of (e); (e) "ventral lock" and internal microsculpture, inset shows single cloven-hoof-like scale; (f) ventral view of uncovered "apical lock". d: distal direction. Arrowheads mark sensilla (black), microsculpture (white) and serration (empty).

sensilla – those in proximal cluster oriented perpendicular to long axis of V1, those in distal cluster oriented parallel to long axis of V1 (Figure 3b). Internal surface of V1 with a system of longitudinal and oblique ravioli-like ribs along ventral margin and apical part, which probably serve to keep the two halves of V1 together (Figures 3d–f, 5a). Internal microsculpture of V1 consisting of cloven-hoof-like scales and corrugations (Figure 3d–f). Expanded base of each V2 with field of short setae externally (Figure 4a). V2 surface near basis with numerous firm knobles, each with sensilla inside (Figure 4a–c). Distal half of V2 with 11 transverse ridges (Figures 2a, 4d). Knobles reduced in apical direction and disappearing completely near most proximal ridge. Ventral edges of each V2 forming a longitudinal rail (rhachis), forming a part of the sliding articulation with aulax of V1 (Figure 4e). No connection between the dorsal edges

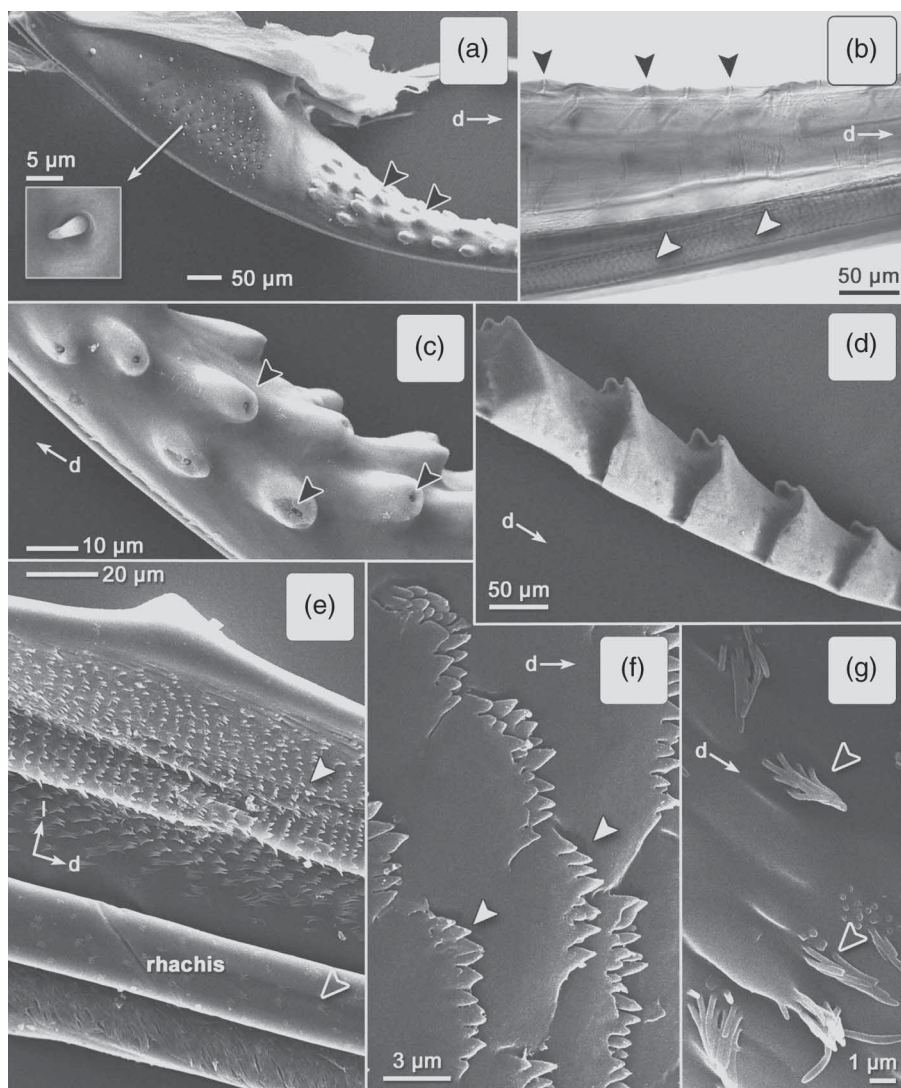


Figure 4. Second cutting valve of *Lestes macrostigma*, scanning electron micrographs (a, c–g) and transmission light micrograph (b): (a) lateral view of the basal part with field of short setae, inset shows single seta; (b) internal view in a middle area with smoothed knobble-like cuticular projection around each sensillum and trans-cuticle canals running to each sensillum; (c) knobbls with sensilla in the basal area, dorsolateral view; (d) dorsolateral view in area of apical ridges; (e) internal surface and rhachis; (f) internal microsculpture consisting of comb-like scales; (g) microsculpture of rachis consisting of finger-like scales. d: distal direction, l: lateral direction. Arrowheads mark sensilla (black), comb-like (white) and finger-like (empty) scales.

of the V2 pair. Internal surface of V2 with comb-like microspinations, surface of rhachis with finger-shaped microspinations (Figure 4b, e–g). Unpaired internal sclerite (piv) small, slightly compressed laterally and connected to bases of both V2 by membranous cuticle (Figure 2b).

Basal plate of ovipositor

Basal plate of ovipositor (Lam) represented by a group of three fused sclerites connecting first valves with sternite of S8 and tergite of S9. Central part of Lam formed by paired gonocoxae of

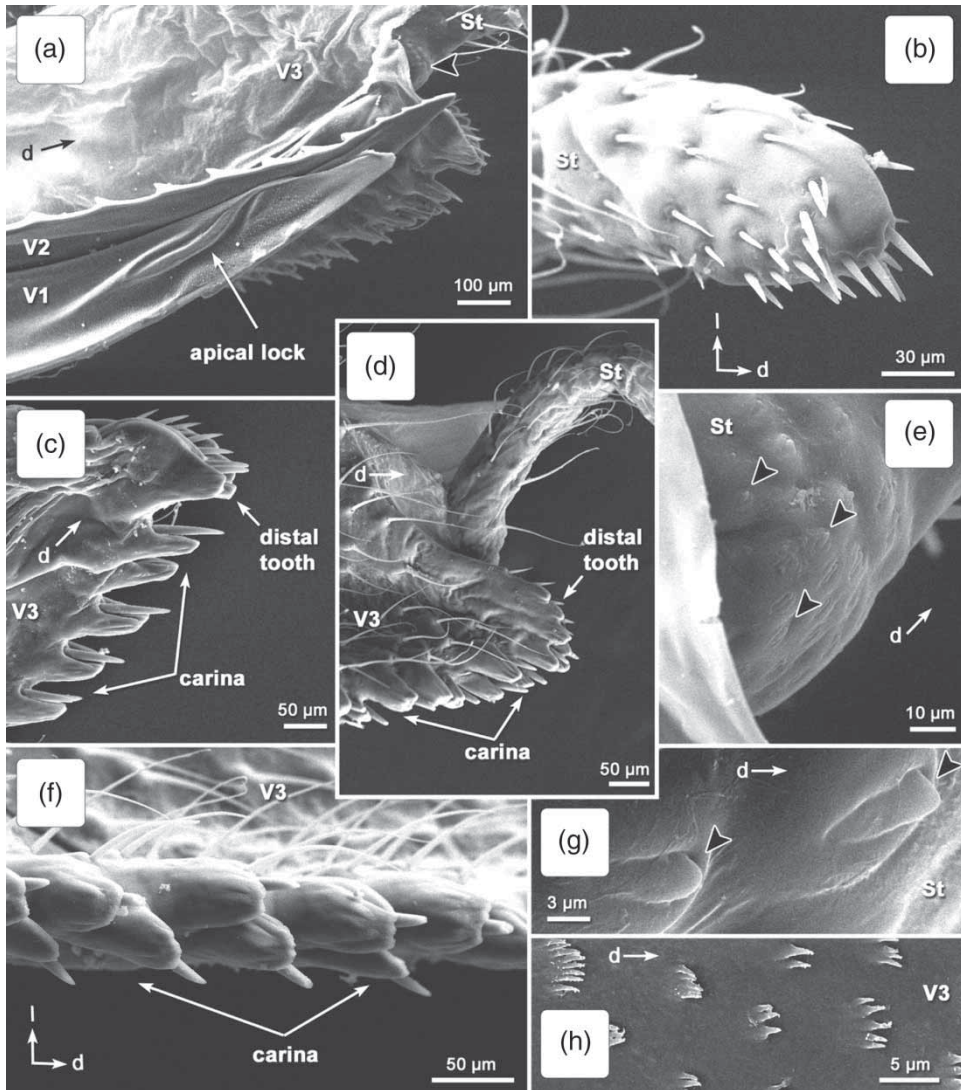


Figure 5. Scanning electron micrographs of sheathing valve and stylus of *Lestes macrostigma*: (a) internal view of apical parts of ovipositor showing valves in resting position; (b) apex of stylus; (c) internal view of apical part of carina; (d) external view of apical part of carina; (e) field of campaniform sensilla on base of stylus; (f) ventral view of carina; (g) two campaniform sensilla of stylus; (h) internal microstructures of sheathing valve consisting of comb-like scales. d: distal direction, l: lateral direction. Arrowheads mark campaniform sensilla.

S8 fused medially. Posterolateral part of Lam comprising paired anterior parts of gonangulum (Ga) (Figures 1, 6b), and slightly movable relative to the central part of Lam. Endoskeletal paired medial and lateral apodemes serving as attachment points of muscle M1 and M2, respectively (Figures 2a, b, 6).

Sheathing valves

Sheathing valves (V3) ensheathing cutting valves laterally in resting position (Figure 5a). Endoskeletal part of each V3 with two apophyses, aAp and pAp, bearing attachments of muscles

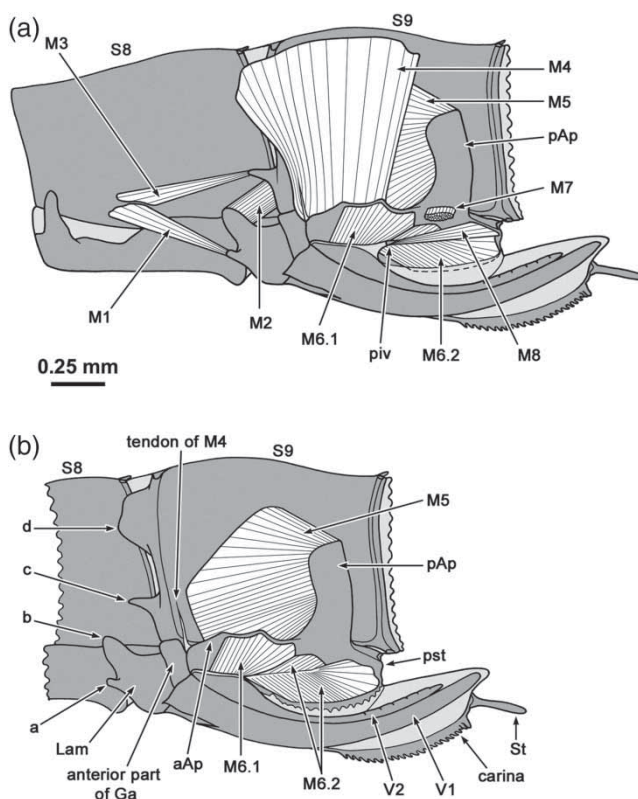


Figure 6. Musculature of the ovipositor of *Lestes macrostigma*, medial view: (a) internal layer; (b) external layer.

M4 and M5, respectively (Figures 2b, 6). External surface of each V3 with scattered long, thin setae laterally, ventral edge of each V3 provided with row of large teeth, with single robust seta just posterior to each tooth (Figure 5c, d, f). Distal tooth largest, with seven robust setae (Figure 5c, d). Teeth of V3 form together a bearing edge, or carina, by which a female leans against an oviposition substrate during egg laying. Movable St above distal tooth of carina, cylindrical, somewhat swollen at basis (Figure 2). Surface of St covered with sparse short thin setae directed apically. Apex of St with short robust setae forming bunch on ventromedial surface (Figure 5b). Field of campaniform sensilla on ventromedial surface of swollen basis of St, oriented parallel to long axis of St (Figure 5a, e, g). Comb-like microspinations on medial wall of V3 (Figure 5h). Paired elongated sclerite (posterior part of gonangulum) situated lateral to V3 and connected to tergite of S9 via fusion and to V3 via highly movable articulation beneath of pAp (Figure 2a).

Musculature

Musculature of ovipositor represented by one unpaired and seven paired muscles (Figure 6). Paired M1 originating on lateral part of 8th tergite and inserting on medial apodeme (a) of Lam. Paired M2 originating on posteroventral edge of 8th tergite and inserting on lateral apodeme (b) of Lam. Paired M3 connecting lateral part of 8th tergite and anteromesal apodeme (c) on anterior carina of 9th tergite. Paired muscles M4 and M5 largest. M4 originating on anterodorsal apodeme (d) and lateral wall of 9th tergite and inserting on aAp of V3. M5 originating on dorsolateral, lateral and ventral parts of S9 and the elongated sclerite and inserted on pAp. Paired M6 divided

into two fascicles, anterior straight M6.1 and posterior fan-shaped M6.2. M6.1 originating on V3 beneath aAp and inserting on basal part of V2 directly. M6.2 originating on V3 beneath pAp and on PS9, inserting on basal part of V2 via tendon, and exhibiting at least three regions of differently oriented muscle fibres. M7, the only unpaired muscle of the ovipositor, running transversely and connecting the two V3 beneath pAp. Paired M8 attaching on either side of internal sclerite (piv) and inserting on V3 beneath of pAp and on PS9.

Oviposition behaviour

The following description is based on observations of successful oviposition, during which egg clutches consisting of several eggs were produced. After copulation, males mainly accompanied their mates in tandem. Oviposition was mostly performed in vertically directed stems, but also in broken and therefore horizontal or oblique directed stems of *Bolboschoenus maritimus* and *Juncus maritimus*, either green, partially dried, or entirely dried for *J. maritimus* only. Height of oviposition sites was significantly lower in *B. maritimus* than in *J. maritimus* (*B. maritimus*: median = 100 mm, $n = 58$, $W = 0.92$, $p = 0.001$; *J. maritimus*: median = 150 mm, $n = 74$, $W = 0.92$, $p = 0.0003$; $U = 1056$, $p < 0.0001$). No underwater oviposition was observed. Shortly after landing on a stem, females performed repetitive movement patterns that resulted in production of an egg clutch. The female bent its abdomen in so as to lean the ovipositor again the plant surface, segments 3–4 and 4–5 both forming a right angle (Figure 1). Before she started to insert her ovipositor in the plant, she touched the substrate with the ovipositor up to six times. These touches may provide information leading to a choice of insertion site (see Lutz & Pittman, 1968). Then ovipositor valves V1 and V2 protruded from V3 and began to move alternately, gradually penetrating more deeply into plant tissue. The position of the ovipositor tip varied somewhat during this time depending on female body adjustment after the ovipositor was definitely positioned (Figure 1): between the mid-legs (*B. maritimus*: 20%, $n = 10$), between the mid- and hind-legs (*B. maritimus*: 60%; *J. maritimus*: 47%, $n = 13$), between the hind-legs (*B. maritimus*: 10%; *J. maritimus*: 40%), or slightly behind the hind-legs (*B. maritimus*: 10%; *J. maritimus*: 13%). During ovipositor insertion the female slightly swung and rotated its abdomen from side to side (Figure 1b). When its cutting valves became entirely inserted in the plant, the female stayed motionless for a of couple seconds, only moving the tip of its abdomen at very low amplitude. The egg was probably laid into the prepared hole during this time. Then the female removed the cutting valves from the hole and walked a few steps backward. She kept her abdomen curved ventrally, but the ovipositor tip lost contact with substrate for a moment. Repetitive movement patterns recurred. The duration of one egg deposition was significantly lower for *B. maritimus* than for *J. maritimus* (*B. maritimus*: median = 5 s, $n = 52$, $W = 0.95$, $p = 0.023$; *J. maritimus*: median = 52 s, $n = 57$, $W = 0.92$, $p = 0.002$; $U = 885.5$, $p < 0.0003$). Oviposition was often carried out on plant stems that already contained egg clutches, and many stems with several egg clutches were observed.

Egg clutch

An egg clutch consists of a row of a few single eggs deposited in one line. Eggs were 1.36 ± 0.07 mm long (L , min = 1.22, max = 1.50, $n = 31$, $W = 0.98$, $p = 0.83$). Of the eggs examined, 8.51% and 21.28% were decayed and parasitized, respectively ($n = 47$). Median distance between successive eggs in a clutch was 2.06 mm (d_1 , $n = 44$, $W = 0.64$, $p < 0.05$). Each successive egg insertion slightly deviated from the previous insertion randomly to the left or to the right relative to direction of plant fibres (median $\gamma = 1.94^\circ$, min = -6.38 , max = 8.46 , $n = 44$, $W = 0.84$, $p < 0.05$) so that the clutch line ran approximately parallel to the longitudinal axis of the plant

stem. Eggs were oriented in plant tissue at angles of $15.37 \pm 3.41^\circ$ to the plant surface (α , min = 8, max = 21, $n = 35$; $W = 0.96$; $p = 0.27$). Lateral deviation of the egg axis relative to the direction of fibres in plant tissue was variable ($\beta = -1.37^\circ \pm 7.19^\circ$, min = -15.00 , max = 12.00 , $n = 35$, $W = 0.96$, $p = 0.28$).

Discussion

Morphology of the ovipositor

The ovipositor of *Lestes macrostigma* belongs to the endophytic type that occurs in all Zygoptera, the anisozygopteran, *Epiophlebia superstes*, and most aeshnids (Asahina, 1954; Matushkina, 2004, 2008; Matushkina & Gorb, 1997; Pfau, 1985; St. Quentin, 1962). The exoskeleton and muscles of the ovipositor have been studied in several lestids, namely *Sympecma paedisca* (Brauer), *L. barbarus* (Fabricius), *L. sponsa* (Hansemann), *L. virens* (Charpentier) and *Chalcolestes parvidens* (Artobolevsky) (Matushkina, 2004, 2007a; Matushkina & Gorb, 1997). All these species, as well as *L. macrostigma*, have the complete muscle set of the ovipositor that comprises seven paired muscles and one unpaired muscle. Muscle M6 shows the most considerable morphological variation among all dragonfly species studied in this regard. Although it is undivided in some dragonfly lineages (*Calopteryx splendens*: Matushkina & Gorb, 1997; *Epiophlebia superstes*: Matushkina, 2008), muscle M6 of lestids is always divided into anterior and posterior parts. Moreover, the posterior part of M6 in Lestinae can be represented by one fascicle in *L. barbarus* and *L. macrostigma*, by two fascicles in *L. sponsa* and *L. virens*, and by three fascicles in *C. parvidens*. As a result of contraction of M6, the valves V2 move relative to the sheathing valves. Most likely, these movements occur during rhythmical penetration of the cutting valves into the oviposition substrate, i.e. during producing of an egg clutch, and can be related to the exact orientation of V2. It can be supposed that the morphological differentiation of M6 may indicate a functional one, so separation of the muscle M6 into different fascicles seems to have some relation to producing of an egg clutch of a particular pattern. Following this reasoning, an egg clutch of *L. macrostigma* is expected to be somewhat similar to those of *L. barbarus* (see also below).

For the first time, cuticle microstructures of the ovipositor in lestids were specially studied using SEM. The ovipositor of *L. macrostigma* is provided with receptors of different shapes. Numerous campaniform sensilla were found on the basis of V1. Arranged in two groups with parallel and perpendicular orientation to the long axis of the valve, they can detect cuticle strains that arise in V1 during substrate penetration. The field of campaniform sensilla on the basis of the stylus on V3 responds to the stylus inclination, which occurs when the ovipositor contacts a substrate. These two fields, located symmetrically on the right and left styli, may function as controllers of spatial characteristics of an egg clutch, as was previously presumed for *L. sponsa* (Matushkina & Gorb, 2002a). At least some coeloconic sensilla on the external surface of the cutting valves have an apical pore that could imply a chemosensory function for them. Robust setae on the apex of the stylus and on the carina of V3 contact the plant surface during egg laying and are probably mechanoreceptors, since they lack any pore on their surface. Several knobbls, serrations and ridges, which were found on the external surface of the cutting valves, probably function in sawing of plant tissues. A row of teeth on the carina of V3 forms a bearing edge in *L. macrostigma*, which functions to hold the female abdomen on the plant surface during plant penetration.

The internal surface of the valves reveals a surprisingly rich microsculpture. Especially noteworthy is the presence of two regions of each V1, the ventral edge and the apical surface, which have peculiar ravioli-like ribs. Located slightly asymmetrically on the two first valves, they form a key-and-lock device that hold the first valves firmly together and are named therefore the “ventral lock” and “apical lock”, respectively. Similar structures on the ventral edge of V1 were recorded

in most of other Zygoptera that have been studied in this regard, as well as in the anisozygopteran, *Epiophlebia superstes* (Klass, 2008). SEM study has shown that unlike the smoothed “apical lock” of *E. superstes*, relief of the “apical lock” in *L. macrostigma* is more distinct and its surface has a complicated microsculpture (Matushkina, 2008).

Oviposition behaviour

The reproductive behaviour and habitat selection of *L. macrostigma* have not been specially investigated so far. Only four plant species were recorded as oviposition substrates of this dragonfly, namely *Bolboschoenus maritimus* (Plattner, 1967), *Schoenoplectus lacustris* (= *Scirpus lacustris*) (Stark, 1980), *Juncus maritimus* (Lambret et al., 2009), and some unidentified species of *Carex* (Martynov & Martynov, 2007). Egg laying of *L. macrostigma* revealed several behavioural traits that are typical for other species of genus *Lestes*. After copulation, *L. macrostigma* remained in tandem and so the female mostly performed oviposition accompanied by its mate. Guarded oviposition is also usually found in *L. sponsa* (Robert, 1958), *L. eurinus* Say (Lutz & Pittman, 1968), *L. unguiculatus* (Hagen) (Bick & Hornuff, 1965), *L. barbarus*, *Chalcolestes viridis* (Grand & Boudot, 2006), *Archilestes grandis* (Rambur) (Bick & Bick, 1970). Female *L. virens* laid their eggs either in tandem or unguarded (Robert, 1958) and females of *L. rectangularis* Say typically oviposited alone (Gower & Kormondy, 1963). Like other *Lestes* species, the female of *L. macrostigma* laid its eggs preferably in vertically directed plant parts (Bellmann, 1987).

Females, whether or not they are in tandem with a male, take a distinctive pose during substrate penetration. Females of European lestids bend their abdomen to a variable degree, so that the cutting valves of the ovipositor are located between the middle leg pair in *Chalcolestes parvidens* (Artobolevsky), between the middle legs to between the middle and hind legs in *L. barbarus* (Fabricius), between the middle and hind legs to between the hind legs in *L. virens* (Charpentier), between the hind legs or slightly behind them in *L. sponsa* (Hanseman), or further beyond the hind legs in *L. dryas* Kirby (Jödicke, 1997; Matushkina, 2007a; Matushkina & Gorb, 2000). It was hypothesized that the degree of curvature of the abdomen in ovipositing females is correlated with stiffness of the substrate and therefore with the force a female can produce during penetration of the plant tissues, i.e. the more curved is the abdomen, the stiffer the plant substrate used for oviposition (Jödicke, 1997). This assumption has been indirectly confirmed by Matushkina and Gorb (2007), who showed that stiffness of the ovipositor decreased in a series of species: *Chalcolestes parvidens* > *L. barbarus* > *L. sponsa* > *L. virens* and that a high positive correlation exists between stiffness of the ovipositor and preferred plant substrates.

In our observations, the position of the ovipositor tip during plant penetration varied in *L. macrostigma* from between the middle legs to slightly behind the hind legs, with the most frequent case being between the middle and hind legs. It seems possible that the actual pose of an ovipositing female depended on local stiffness of different plant parts, and therefore the observed variability of a female's pose can be caused by heterogeneity of mechanical properties of the substrate. Nevertheless, the pose of ovipositing females of *L. macrostigma* resembled most often that in *L. barbarus* and in *L. virens*. In this regard, it is interesting that *L. barbarus* and *L. macrostigma* are the only European dragonfly species which lay their eggs in the sea rush, *J. maritimus* (review in Matushkina & Gorb, 2002b).

Egg clutch

Information about egg clutches in *L. macrostigma* is scanty. Martynov and Martynov (2007) described individual egg position that accords well with the present results but an egg clutch that seemed to consist of two parallel rows of eggs, with one row slightly out of alignment with the

other. This pattern resembles superficially a zigzag egg clutch of some dragonflies, with each egg positioned at the corner of the zigzag clutch (“simple zigzag pattern” following Matushkina & Gorb, 2000). However, accurate analysis of the related illustration of an egg clutch (Martynov & Martynov, 2007, figure 3, p. 188) reveals that distances between successive eggs laid in alternate right and left directions of the zigzag are unequal – distance to the left is almost twice as large as the distance to the right. Therefore we assume that this pattern actually represents two closely deposited linear clutches of eggs laid from above to below, one after the other.

In contrast, Aguesse (1968, figures 5.3, 5.4, p. 21) illustrated very unusual two-egg sets of *L. macrostigma* where the second egg was deposited in the same hole over the first egg. Although the author provided no additional text description, the clutch line seems to be also linear.

Our results have shown more variability of most of clutch characteristics of *L. macrostigma* from stems of *J. maritimus* as compared with other lestids studied so far (Matushkina & Gorb, 2000). Nevertheless, the longitudinal axis of the clutch line is more or less parallel to the plant fibres, and we did not find any case of oviposition of more than one egg in one incision. Hence, the egg clutch of *L. macrostigma* can be tentatively considered to follow a “simple linear pattern”. Among the lestids studied, simple linear clutches are peculiar to *L. barbarus* (Matushkina & Gorb, 2000), *L. rectangularis* Say (Gower & Kormondy, 1963) and probably to *L. dryas* and *L. virens* (see review of Jödicke, 1997). Interestingly, one of the most constant parameters of an egg clutch of *L. macrostigma*, the distance between successive eggs, is almost equal to those in *L. barbarus* (mean = 2.32 versus 2.39 mm, respectively) (Matushkina, 2007b). Due to the limited number of clutches studied, it is still not clear whether or not the parameters of an egg clutch of *L. macrostigma* might be more constant in some “optimal”, relative homogenous substrate, and consequently whether or not the process of egg deposition by *L. macrostigma* can be represented by a highly stereotyped sequence of movements like that known for some other lestids (*L. barbarus*, *L. sponsa*, *C. parvidens*: Matushkina & Gorb, 2000, 2002a). As with the pose of a female of *L. macrostigma* during plant penetration, the variability of egg clutch parameters probably can be explained as local disturbance caused by structural heterogeneity of the substrate.

Future research

In 2009, the second author initiated a project, the main goal of which is to study the biology of *Lestes macrostigma* and to provide recommendations for possible protection of this dragonfly, which is threatened over a wide geographic area. Key subjects of this project are the reproductive biology of *L. macrostigma*, its habitat and oviposition site selection, and the influence of temperature and salinity on larval development. Those studies represent an initial step within the above framework. The results of preliminary field observations suggest to us that some peculiarities of egg laying behaviour can be related to properties of the oviposition plant. If so, it can be expected that a broader correlation exists between the oviposition characteristics of a dragonfly (e.g. duration and rate of egg deposition, security of a female during the egg laying, oviposition success, size and pattern of an egg clutch) and the characteristics of its oviposition plant (e.g. its morphological and mechanical properties and the availability and frequency of suitable substrata in a habitat). We also suspect that in the future both field and laboratory experiments could make it possible to estimate the benefits of the use of some kinds of oviposition plant by this dragonfly species. Such data might clarify the causes of the disjunct distribution of *L. macrostigma* in Europe and contribute to proper conservation management.

Acknowledgements

We would like to thank Alex Gumovsky who provided specimens of *Lestes macrostigma* for morphological studies and Viktor Fursov who helped with light microscopy (Schmalhausen Institute of Zoology, Kiev, Ukraine). Reinhard Jödicke

(Westerstede, Germany) and Michael L. May (Rutgers University, USA) are sincerely thanked for kindly improving of the text. We also would like to warmly thank the Amis des Marais du Vigueirat for the access to the protected area and the Research Center of Tour du Valat for the use of the micrometric stereo-microscope. This study was partly supported for MN by a grant from the German Research Foundation (DFG, grant 436 UKR 17/27/06).

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